REGENERATION OF GONAD TUBULES FOLLOWING EXTIRPATION IN THE SEA-CUCUMBER, THYONE BRIAREUS (LESUEUR)

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Several accounts infer that the extensive powers of visceral regeneration in the holothurians may extend to the gonads (Torelle, 1909; Deichmann, 1921; Bertolini, 1932) although they give neither precise data regarding the portion of the gonad involved nor even a gross analysis of the regenerated tissues. The present study considers the general problem of gonad regeneration in the holothurians with special reference to three specific points, namely, (1) the extent of the capacity to regenerate gonadal tissue, (2) the presence or absence of germ cells within regenerated tubules and (3) the possible origin of any germ cells found within them.

Materials and Methods

Previous experience demonstrated (Kille, 1931) that *Thyone briareus* (Lesueur) is an extraordinarily good holothurian for experimental purposes. In contrast to the genus *Holothuria* which eviscerates through a tear in the cloaca, this genus casts off the entire anterior end of the body whenever it autotomizes the digestive system. With these parts lost, the animal is nothing but a dermo-muscular sac containing only a cloaca, respiratory trees, gonads, and mesentery. If left to itself, the circular body muscles at the extreme anterior end contract strongly so as to close off the body cavity from the sea. A healing process soon makes this closure permanent. However, if one makes a longitudinal cut at the anterior end equivalent to about one-fifth the length of the animal, many specimens may be turned inside out following their evisceration. In such a position the gonad tubules and the mesentery hang freely in the water. (Fig. 1, *A, B, C*). With the animal in this position, operations on the gonad are possible. Most specimens can then be turned right side out with little difficulty. If the animal is returned to running water, a high percentage survive the operation (Kille, 1937). In some cases the circular muscles of the body wall contract so

strongly that the animal cannot be turned right side out. Such individuals do not live more than a day or two.

The Reproductive System in Thyone briareus (Lesueur)

A thick tuft of gonad tubules is found in each side of the dorsal mesentery as seen in Fig. 2. They originate from tissue localized within the mesentery in a region near the dorsal body wall (Fig. 2, gonad-basis) and about midway between the mouth and the anus (Fig. 1, *C*). These tubules empty their products into a common chamber located within the dorsal mesentery (gonad-basis chamber, Fig. 2). Anterior to the tubules this cavity continues as the lumen of a gonoduct which runs through the dorsal mesentery to open exteriorly at a point between the bases of the two dorsal tentacles. Each right and left half of the

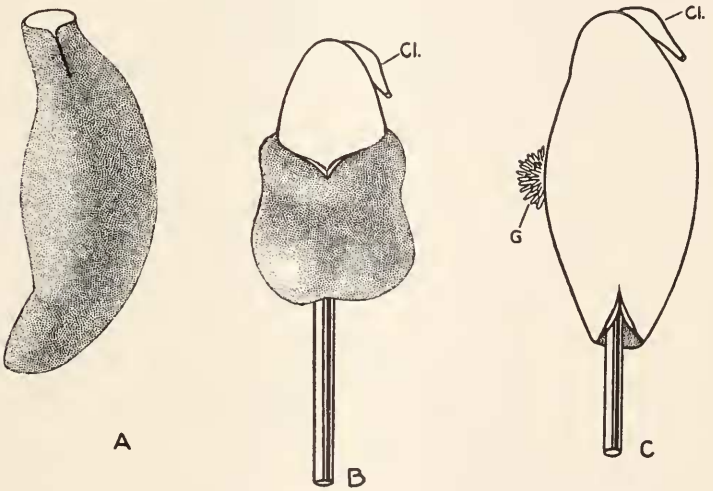

Fig. 1. *A, Thyone* immediately after evisceration with a short longitudinal slit through the body wall at the anterior end (ventral surface to the right); *B, Thyone* being turned inside out with the aid of a blunt glass rod; *C,* specimen turned completely inside out. *Cl, cloaca;* G, gonad. (Mesenteries and musculature of body-wall omitted.)

Thyone gonad consists then of (1) a lateral portion of a chamber located within the dorsal mesentery (the gonad-basis) and (2) a mass of tubules which take their origin from the ventro-lateral wall of the chamber (gonad tubule, Fig. 2). The right and left tufts of tubules are symmetrically developed, there being but little variation in the number and size of tubules between the two sides. Within each tuft, however, one can distinguish tubules in various stages of development. The ex-

treme anterior ones are small, unpigmented, translucent, immature tubules. Such a condition has been described for many genera (Théel, 1901; Mitsukuri, 1903; Haanen, 1914; Deichmann, 1930, etc.). Posterior to these is a group of tubules which grade into the slender, more mature tubules possessing pigmented, opaque walls, rounded extremities and greater length. These long, opaque tubules make up the bulk of the gonad, yet the shorter, anterior tubules comprise at least a fifth of the total number of tubules present. It should also be mentioned that

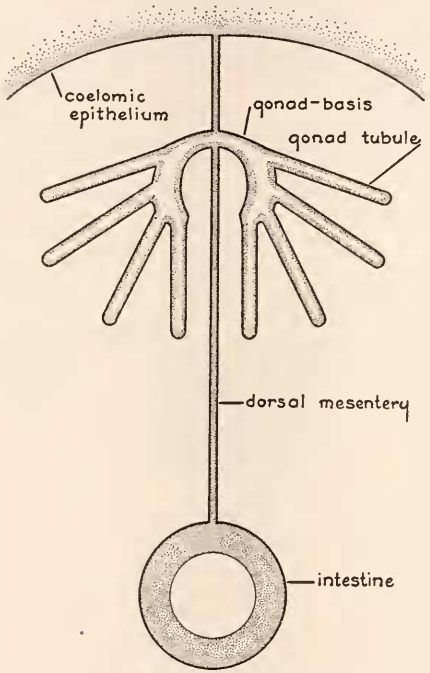

Fig. 2. Diagrammatic representation of a cross-section through a piece of the dorsal body wall with the attached mesentery to show the relationship existing between body wall, mesentery, gonad and intestine.

in the larger specimens collected during July and August there is an indication that posterior to the tuft of tubules still older tubules may have been lost.

In the medium-sized specimens used in these experiments, one can say that the larger the specimen, the greater is the mass of the gonad for the tubules are not only larger but their number is greater (see Table I). Within two larger specimens whose body volume was approximately 140 cc., the count for the female was 400 tubules while the male possessed 1035.

All these data indicate that there is a continued anterior proliferation of the tubules from the gonad-basis. Histological analysis of the tubules supports this conclusion. The posterior tubules show various stages in gametogenesis though there are fewer mature germ cells present than in those tubules more anterior, which may be packed with well-developed gametes. The most anterior tubules, however, show no ripe gametes but enormous numbers of germ cells in the early stages of gametogenesis. At the present time, I am not prepared to give an account of the seasonal variation in the gonad for all material discussed in this paper, whether normal or experimental, was collected in the months of July through October.

Table I

The total number of tubules found within a series of eleven specimens closely graded as to size.

Size of Specimens (Volume in cc.)	Approximate Number Tubules in Each Tuft ♀	♂
4	130	
4.5	110	
5.8	107	200
6.6	150	
8.0	160	
10.0		250
12.0	185	
13.0	170	
14.0	200	
19.0	170	

Results of the Removal of the Gonad Tubules either with or without the Gonad-basis

Since it is possible that part of the gonad-basis is torn away with the gonad tubules whenever they are entangled in the digestive system during the process of evisceration, two types of operations were carried out. In Group I, the gonad tubules and the entire gonad-basis were extirpated from 6 female and 6 male specimens of medium-size. These were collected at Woods Hole in early August. In Group II, composed of a similar group of specimens, the gonad tubules were torn away from the gonad-basis by scraping the gonad area with the blunt back of a scalpel. This was done in order to produce a condition similar to that which occasionally exists after tubules have become entangled in a digestive system that is being eviscerated. Though this seldom happens in *Thyone briareus* (Lesueur) at this season, it frequently occurs in certain Holothuria when the gonads are well-developed.

In both groups, the ability to feed indicated that the new digestive system was established within 15 to 22 days, which is the average period of time required for unoperated specimens (Kille, 1935) as well as for the controls to these experiments. Evidently this unusual procedure for a coelomic operation has no injurious effect. None of Group I, however, showed any signs of gonad regeneration. Eleven specimens were killed for examination at various intervals after the operation, ranging from 46 to 129 days. The other remaining specimen of the

Fig. 3. Ventral view of the testes within a *Thyone* 63 days after the main mass of the gonad had been scraped away. For size comparison, the proximal quarters of 5 mature tubules of the original right testis are included on the reader's left.

group was in poor condition at the end of the experiment and was discarded. In all cases the new digestive system and its associated structures were well developed but the cut end of the gonoduct had healed blindly.

On the other hand, the gonad-basis of each specimen in Group II proliferated either a right, or a left, or a right and a left tuft of miniature tubules. Evidently all of the very smallest tubules of the original group were not always rubbed off for some of the newly proliferated groups showed a few tubules so much larger and longer than the rest

that it was clear that these belonged to the original gonad mass. Such groups were thus composed of a large number of newly proliferated tubules and, in addition, a smaller number of tubules which were present before the operation (Fig. 3, left gonad). This assumption is supported by the microscopic examination of a number of specimens which were killed immediately after the operation. In some cases, one could identify very minute tubules which remained attached to the remnant of the gonad-basis. At least this remnant must certainly remain even after the most violent self-eviscerations of holothurians.

The method of operation, however, frequently resulted in the removal of all the original tubules. Such an operation would easily account for those newly regenerated tufts which are like the right testis in Fig. 3. All the regenerated tubules are small and equal in development. This condition stands in sharp contrast to what is found in a normal male of equivalent size and season. Typically there would be only a fourth of this number of minute tubules and they would show a graded series as to length. Further evidence that these tubules arose at the same time is furnished by their histological similarities. In each tubule, the germ cells are packed rather solidly within the tubule. In contrast to this, the longer tubules as seen in the left testis (Fig. 3) possess a germinal epithelium surrounding a definite central lumen.

The Origin of the Germ Cells within the Newly Proliferated Tubules

Since the microscopic examination of the newly proliferated tubules showed that they were packed with cells which were identical cytologically with the primordial germ cells found within the tubules of the normal gonad, a careful study was made to determine the origin of these cells. Sections were prepared of normal gonads and of the gonad remnants left within operated but unregenerated specimens. Special attention was given to the anterior region of the normal gonad where the immature tubules occur. Here the small, club-shaped tubules appear to be diverticula of the wall of the gonad-basis. Anterior to these, one finds a few, minute, knobbed protuberances of the wall, each of which possesses a solid core of germ cells. Apparently, each core originates from an independent center within the wall of the gonad-basis, for just anterior to the protuberances separate nests of germ cells are found in those tissues. In view of their size and position, it is not surprising that they have never been described in this species. These nests of germ cells are evidently the equivalent of the "genital stolon" which is quite conspicuous in the dorsal mesentery of some holothurians (Théel, 1901;

MacBride, 1906) and from which the gonad-basis, gonad tubules and germ cells arise.

Examination of remnants of the gonad-basis left within operated animals shows that nests of germ cells may or may not be left on either side as a result of the operation performed upon Group II. This would seem to correlate with the fact that new tubules packed with germ cells may be proliferated on either or both sides following such operations. It is assumed then that such residual nests of germ cells are the source of the germ cells found within the newly proliferated tubules. Though none of these 12 animals failed to proliferate tubules on at least one side, one would expect this to occur occasionally if this assumption is true.

Discussion

Since only a small part of the gonad-basis is ever lost when the gonad tubules are torn out during a process of visceral autotomy, these results show that an eviscerated holothurian does not merely mature certain small, residual tubules but may actually proliferate an entirely new set of gonad tubules. The removal of the original set of tubules is, in reality, a removal of only that part of the gonad in which a portion of the germ cells are maturing. The remnant of the gonad-basis possesses a store of primordial sex cells which are then contributed to the new tubules as they are proliferated at a rate probably four times the normal. It is a case similar to that reported in vertebrates where growth of accessory or residual gonadal tissue may follow incomplete gonadectomy. It is apparent, therefore, that true "regeneration of the gonad" does not occur but merely a proliferation of tubules from a growth zone or area of the gonad-basis.

In a period of 4 months, only a capacity for healing is demonstrated by the tissues of the gonoduct or of the gonad-basis when germ cells are absent. That the proliferation of tubules by the gonad-basis does not occur in the absence of germ cells is suggested by the facts that (1) a tubule stump (which contains no germ cells) never produces a new tubule, and (2) among the new proliferations, no sterile tubules have ever been found. It will also be noted in Fig. 3 that the tubules take their origin from an extremely localized area of the gonad-basis. This manner of origin suggests a dependence upon a restricted group of cells rather than upon a general capacity of cells widely distributed throughout the gonad-basis. It is of interest in this connection to note that in the chicken, Willier (1937) has obtained by means of chorio-allantoic grafting, male-like sex cords even though no primordial germ cells are present. A sexual gland without germ cells may also develop

in the urodele under certain conditions (Humphrey, 1928). Furthermore, Geigy (1931) has shown that in *Drosophila* the formation of a gonad is not dependent upon the presence of germ cells. By means of ultra-violet radiation he destroyed the primitive sex cells in the egg yet he obtained sterile ovaries and testes.

This dependent relationship of the origin of tubules upon the presence of germ cells in *Thyone* may be more apparent than real. A small group of cells at the anterior limit of the gonad area may retain a capacity for tubule formation whereas cells at more posterior levels do not. The relation then might be one of position only, the removal of the germ cells which are in this region invariably involving a loss of these tubule-forming cells. Microscopically there is no evidence for such a differential capacity among the cells of the gonad-basis. The only striking histological difference along its antero-posterior axis is the presence or absence of germ cells.

These results raise an interesting question relative to those holothurians such as *H. parvula* which undergo transverse fission in nature. In these species the gonad is located far anterior and therefore it is retained by the anterior portion. If the regenerative capacity of this genus is as limited as that of *Thyone,* then the posterior portions must continue their reproduction entirely by asexual means. While the occurrence of fission has frequently been reported in certain species of holothurians (Crozier, 1917; Deichmann, 1921; Kille, 1936), there has been no demonstration that it is the sole means of reproduction for certain individuals sterilized by the plane of a previous fission. Deichmann has reported (1921) "feebly developed" gonads within regenerating portions of *Holothuria* (*Actinopyga*) *difficilis.* In a recent communication to the author this investigator states that these gonads were seen only within anterior portions. There is then no report in the literature that the posterior portions ever develop a gonad. The absence of any such natural evidence for the regeneration of the gonads from other tissues coupled with the data obtained through this direct experimental approach makes a strong case for the segregation of tissues with germinal potencies within a highly restricted region of the dorsal mesentery.

As Stolte has recently (1936) pointed out, little is known concerning the cellular basis of regeneration within the echinoderm. Zirpolo (1928) believes there is a widespread totipotency among cells throughout the body of *Asterias.* On the other hand, studies on the genera *Stichopus, Holothuria* and *Thyone* leave us with only one established case to support such a generalization for Holothuria, namely, the formation of a new gut epithelium from the cells of the mesentery in *Stichopus regalis* (Bertolini, 1930). In all other instances new tissues apparently

arise from remnants of the old with the one possible exception of the anterior gut epithelium within a regenerating *Thyone* (Kille, 1935, p. 93). Since holothurians show an unusual capacity for regeneration there has been a tendency to assume that the tissues (or a tissue) of this group possess a totipotency throughout the entire animal. In light of the evidence, this assumption is unwarranted. Apart from the problem of organization, the unusual restorative powers are simply evidence of a normal capacity for cellular proliferation or migration and the maintenance of differentiation under new conditions.

Summary

(1) The gonad in the sea-cucumber consists of two major portions: (*a*) a group of tubules in which germ cells are maturing and (*b*) a basal portion, the gonad-basis, from which these tubules arise. Failure to recognize this has probably led to the incorrect statements that these forms "will regenerate the gonad."

(2) In order to test the extent of the capacity to regenerate gonadal tissue, a partial or a complete gonadectomy was performed upon *Thyone briareus* (Lesueur). The gonad was exposed by turning the animal inside out after an induced autotomy of its digestive system and anterior end.

(3) That this procedure had no very injurious effects is shown by the fact that approximately 95 per cent of the operated animals survived. These and the controls regenerated the autotomized parts as rapidly as did eviscerated but unoperated animals.

(4) There was no regeneration of gonadal tissue following complete gonadectomy.

(5) If only the tubules were removed, new tubules might arise from a restricted, anterior region of the gonad-basis at a rate four times the normal (at this season).

(6) The only histological feature that distinguished this productive region from the rest of the gonad-basis was the presence of nests of germ cells.

(7) The possibility that tubule formation is dependent upon the presence of germ cells was suggested by still other facts. No sterile tubules were ever found among the regenerated tubules. Furthermore, the sterile proximal portion of a tubule which sometimes remained after a tubule had been torn out, did not give rise to a new tubule. Finally, when the tubules were roughly torn out, that portion of the gonad-basis containing the germ cells might or might not be lost and this is correlated with the fact that after such treatment, a *Thyone* may regenerate either a right or a left, or a right and a left tuft of tubules. It is

granted, however, that such evidence is not conclusive. Germ cell origin and tubule formation may be independently related to a totipotence possessed by only a few cells in a restricted portion of the gonad-basis for which these experiments provide no test.

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